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EVALUATION OF THE ACUTE TOXICITY OF FOUR WATER-IN-OIL EMULSION HYDRAULIC FLUIDS

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Melvin E. Andersen

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As a part of the Submarine Damage Prevention program, four commercial water-in-oil emulsion fluids were evaluated for use in high pressure submarine internal hydraulic systems. The water-in-oil emulsion class of compounds consists of stable emulsions of micronic or submicronic water droplets suspended in a high quality petroleum base oil. These fluids also contain special additives to give the final product lubricity, corrosion protection properties, emulsion stabilizers, and resistance to bacterial and fungal growth. The most significant exposure routes for hydraulic fluids are expected to be dermal, due to spills or leaks, and aerosol inhalation, from pressurized system leaks. The studies conducted included eye and skin irritation, skin sensitization, oral and dermal toxicity, and aerosol inhalation. They also provided data to compare the short-term exposure effects of these various emulsions. None of the tested compounds were toxic by the oral or dermal routes of administration. The viscous nature of these emulsions limited the aerosol concentrations which could be tested. At the maximum attainable concentrations, no deaths or toxic effects were noted in the exposed animals. All of the materials were mildly irritating to the eyes but not to the skin. One of the materials tested had a slight potential to sensitize treated animals.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted in the Toxic Hazards Research Unit, Northrop Services, Inc. – Environmental Sciences. This document serves as a final report on the toxicity of four water-in-oil emulsion hydraulic fluids. The research described in this report began in July 1986 and was completed in April 1987. It was performed under U.S. Air Force Contract No. F33615-85-C-0532. Melvin E. Andersen, Ph.D., served as a Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory. The study was sponsored by the U.S. Navy under the direction of CAPT David E. Uddin, MSC, USN.

This work was supported by the Naval Medical Research and Development Command Task MR04122010006. The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of Navy or the Naval Services at large.

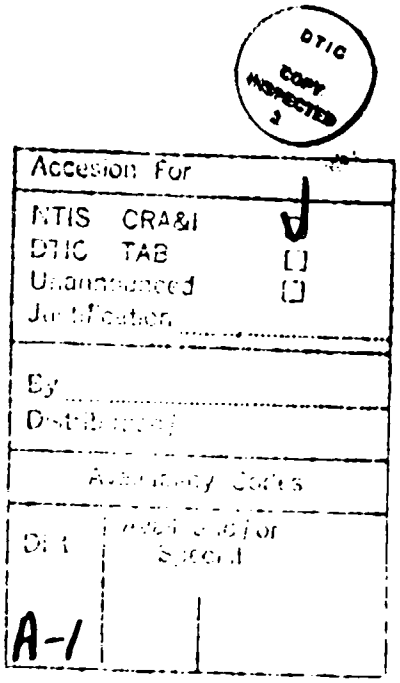

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SECTION 1

INTRODUCTION

As part of the Submarine Damage Prevention Program, the Navy was interested in evaluating four commercial water-in-oil emulsion fluids for use in submarine high-pressure, internal hydraulic systems. The Navy Medical Research Institute/Toxicology Division (NMRI/TD) requested the Toxic Hazards Research Unit (THRU) to conduct a toxicological evaluation of these fluids as part of the process of determining their suitability for shipboard use. The water-in-oil emulsion class of compounds consists of stable emulsions that contain 40% water homogeneously dispersed as micronic or submicronic size droplets in a 60% continuous oil phase. The oil phase is formulated from a high-quality petroleum-based oil compounded with special additives to give the final product additional lubricity, corrosion protection, emulsion stability, and resistance to bacterial and fungal contamination.

The most significant exposure routes for hydraulic fluids are expected to be dermal, due to spills or leaks, and aerosol inhalation, from pressurized system leaks. The following studies, which reflect these potential routes of exposure, include eye and skin irritation, skin sensitization, single-dose oral and dermal toxicity, and aerosol inhalation. Species and sex of animals selected for the acute toxicity tests conform to the requirements of the Environmental Protection Agency.

The purpose of the studies was to develop data that can be used to compare the acute exposure effects of the four water-in-oil hydraulic fluids.

SECTION 2

MATERIALS AND METHODS

TEST AGENTS

The four water-in-oil emulsions supplied by the NMRI/TD, including the various Navy codes, are listed below:

NMRI/TD No.	Supplier	Trade Name
6049-1	Quaker Chemical Corporation	Quintolubric 958 30w
6049-2	Mobil Oil Corporation	Pyrograud A-443
6049-3	E.F. Houghton and Company	Houghto-Safe 5047F
6049-4	Sun Refining and Marketing Co.	Sunsafe F

Pertinent Physical Properties

	6049-1	6049-2	6049-3	6049-4
Boiling pt. (°C):	100.00	100.00	106.70	100.00
Sp. gravity (H_2O = 1):	0.96	0.92	0.92	0.92
% Volatiles by vol.:	------	------	30-60	39.00
pH:	8.00	9.50	9.00	7.20
Appearance:	milky white fluid	milky white fluid	milky white fluid	milky white fluid

TEST AGENT QUALITY CONTROL

A Beckman Acculab 4 was used to obtain infrared (IR) spectra of the four materials; the four samples had similar spectra (Figures 1 and 2). In addition, each fluid was tested for ethylene glycol content (the most toxic substance thought to be present in the test materials). A Varian 3700 gas chromatograph equipped with a flame ionization detector and a 49-m methyl silicon fluid fused capillary column was used in conjunction with a Hewlett-Packard 3388 computing integrator, which measured peak areas and recorded chromatograms. Ethylene glycol standards were prepared in deionized water and injected under the same conditions as the test samples. The results of the ethylene glycol analysis are provided in Table 1.

TABLE 1. SUMMARY OF ETHYLENE GLYCOL CONTENT IN FOUR WATER-IN-OIL HYDRAULIC FLUIDS

Sample	% Ethylene Glycol, v/v
6049-1	2.27
6049-2	2.20
6049-3	2.13
6049-4	1.21

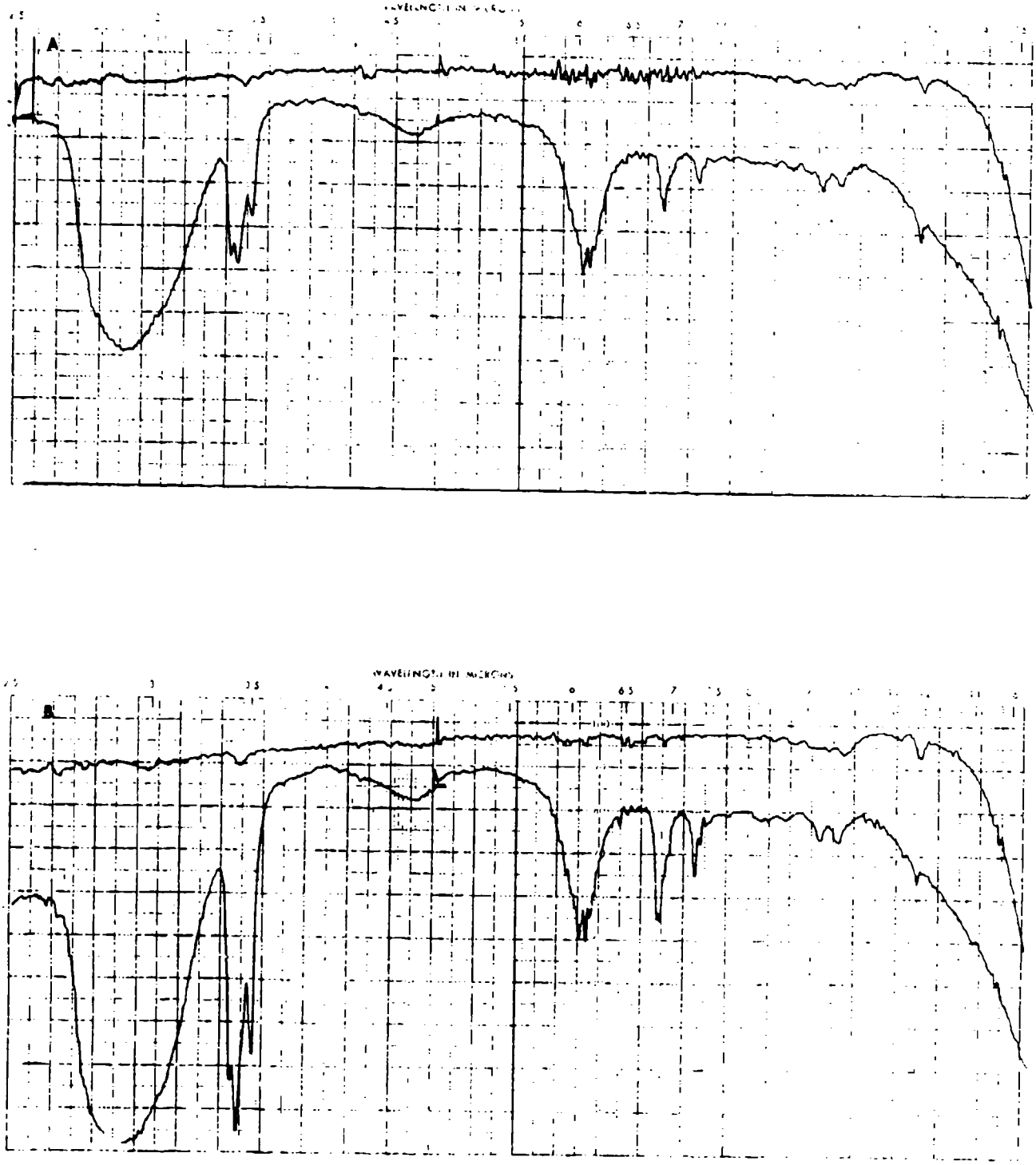

Figure 1. Infrared spectra of (A) Quintolubric 958 (6049-1) and (B) Pyrograud A-443 (6049-2).

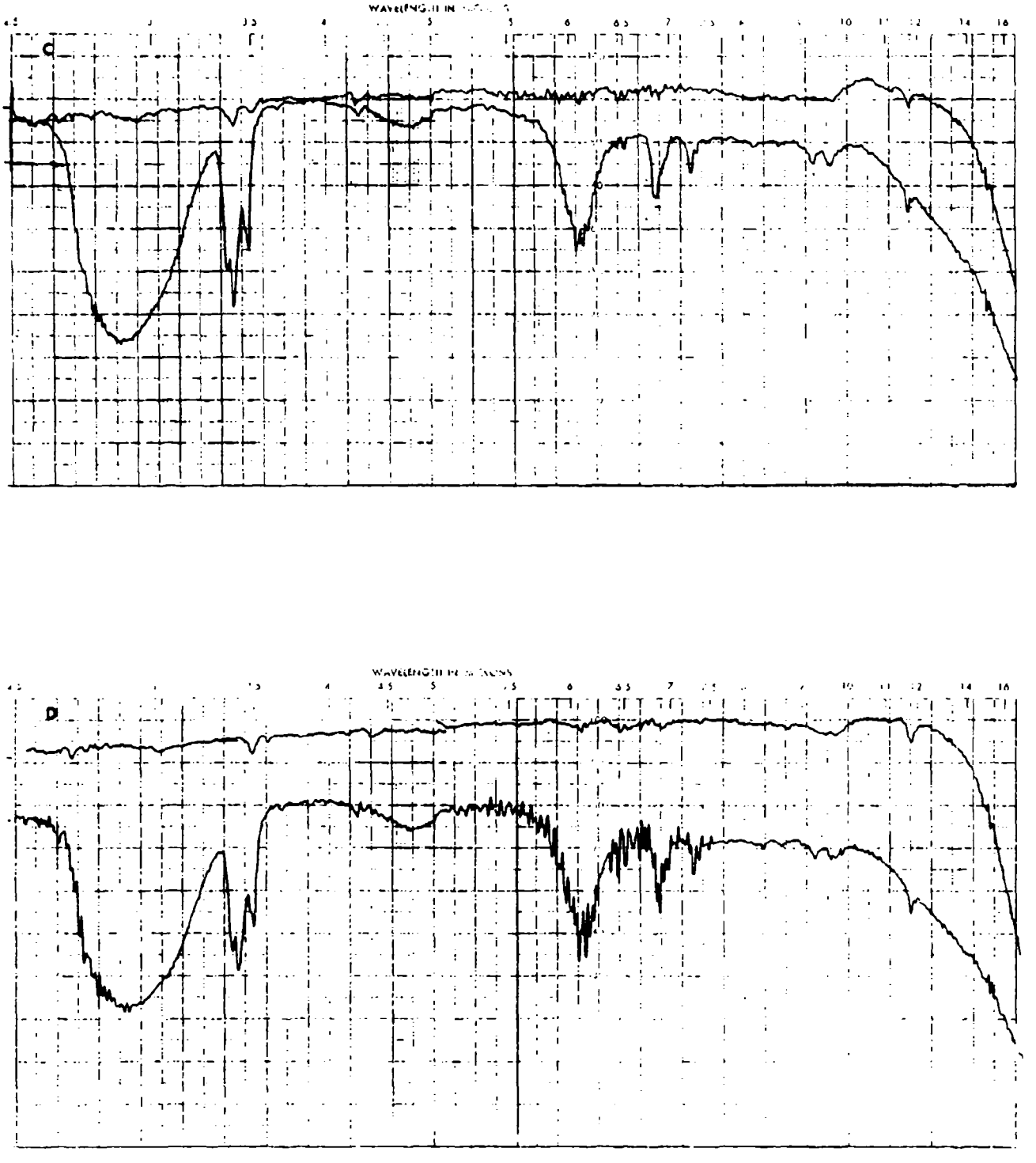

Figure 2. Infrared spectra of (C) Houghto-Safe 5047F (6049-3) and (D) Sunsafe F (6049-4).

ANIMALS

Male and female Fischer-344 (F-344) rats weighing 150-250 and 125-250 g, respectively, were purchased from Charles River Breeding Labs, Kingston, NY. Male Hartley guinea pigs weighing 300-650 g were purchased from Murphy Breeding Labs, Plainfield, IN. Male and female New Zealand white rabbits weighing 2-3 kg were purchased from Clerco Research Farms, Cincinnati, OH. The animals were randomized upon receipt according to a regimen prepared by the Northrop Services, Inc., Biometrics Section. All animals were shown to be in good health following a two-week quarantine period. Rats were group housed (three per cage) in clear plastic cages with wood chip bedding. The rabbits and guinea pigs were housed individually, the guinea pigs in plastic cages with wood chip bedding and the rabbits in wire-bottom stainless-steel cages. Water and feed (Purina Rabbit Chow #5320, Purina Formulab #5008 for rats, and Purina Formulab #5025 for guinea pigs) were available *ad libitum*, except during the inhalation exposure period and when the rats were fasted for 16 h prior to oral dosing. Animal room temperatures were maintained at 21°-25°C except for a two-day period post-inhalation exposure when the building heating system failed and the temperature dropped to 16°C. Some animals showed signs of stress from the temperature drop, such as shivering and diarrhea. The light/dark cycle was set at 12-h intervals.

ORAL TOXICITY

Sixteen hours prior to the administration of the oral dose, five male and five female F-344 rats, age 8 weeks, were fasted. Each rat was weighed just prior to oral gavage dosing and a 5 g/kg dose was administered. Each rat received a volume of 0.01 milliliters per gram of body weight. Test materials were diluted with corn oil that had a peroxide level of 1.07 meq/kg, well within the limits set by our laboratory. Surviving rats were weighed at 1, 2, 4, 7, 10, and 14 days post-exposure and signs of toxicity recorded. On the 14th day post-exposure, rats were sacrificed and gross pathology was performed. Any gross lesions noted in orally dosed animals at necropsy were sampled for histopathologic examination. An undosed control group was maintained for body weight measurements only. A repeated-measures test was used to compare test animal body weights against controls (Barcikowski, 1983).

DERMAL TOXICITY

Twenty-four hours prior to dosing, the back and sides of five male and five female New Zealand white rabbits weighing 2-3 kg were clipped. The undiluted dose of 2.0 g/kg was applied to the back of the rabbits and spread evenly to both sides. The dose was kept in place by applying an eight-ply gauze patch over the liquid. A clear plastic wrap was then applied over the entire midsection and was held in place with Vetrap® and elastoplast tape. The dose was kept in contact with the rabbit skin for 24 h. After 24 h, the tape, plastic wrap, and gauze were removed and the residual test material was wiped from the animal. Animal body weights were recorded on days 1, 2,

4, 7, 10, and 14 post-treatment. Signs of toxicity and mortality were monitored, and gross pathology was performed at the termination of the study.

EYE IRRITATION

Nine New Zealand female white rabbits, weighing 2-3 kg, were examined with fluorescein stain prior to use to ensure absence of lesions or injury. A topical anesthetic (Alcaine; Proparacaine HCl 0.5%) was instilled in the eyes, treated and control, of all rabbits approximately 2 min prior to application of the test material. One tenth of a milliliter of the test material was applied to one eye of each of the nine albino rabbits. The opposite eye was left untreated and served as the control. The treated eye of three rabbits was flushed with lukewarm deionized water for 1 min starting 30 s after instillation. The eyes of the remaining six rabbits were not flushed. Examination for gross signs of eye irritation were made at 1, 24, 48, and 72 h following treatment. Irritation was scored according to the method of Draize et al. (1944; see Appendix 1), in which the total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctiva.

SKIN IRRITATION

Six New Zealand white female rabbits were clipped on the back and sides 24 h prior to dosing to allow for recovery of the skin from any abrasion resulting from the clipping. The test agent was applied in the amount of 0.5 ml to a designated patch area and was covered by a 3-cm square of surgical gauze two single layers thick. The gauze patch was held in place with strips of surgical adhesive tape and the entire shaved area covered with dental dam and secured with an elastic bandage and adhesive tape. The patches remained in place for 4 h, at which time all wrapping was removed and the excess material wiped from the skin. The test areas were then evaluated for irritation using the Draize Table (Draize et al. 1959; see Appendix 2) as a reference standard. Additional evaluations were performed at 24, 48, and 72 h. The total score of the four observations for all rabbits was divided by 24 to yield a primary irritation rating, which was interpreted using the National Institute for Occupational Safety and Health (NIOSH) skin test rating (see Appendix 3).

SENSITIZATION

Prior to the start of the study, three male Hartley guinea pigs were clipped on both flanks and treated with the test material so as to identify a non-irritating concentration to be used for the sensitization study. After the proper concentration was determined, 10 male guinea pigs were treated on the clipped left flank with 0.1 ml of the diluted test material in mineral oil to determine the baseline irritation response. Hypersensitive guinea pigs were eliminated from the study.

The site of the sensitization test was an area just behind the shoulder girdle. This site was clipped with an Oster® animal clipper and depilated with a commercial depilatory[1] 4 h prior to

treatment. A Vetrap® frame with a 1.5- x 1.5-cm opening was affixed to the guinea pig at the site of the depilated area. One-tenth of a milliliter of the test material was topically applied to the test area and covered with gauze, dental dam, and adhesive tape. This was done on Mondays, Wednesdays, and Fridays until a total of four sensitizing treatments were applied and evaluated. Along with the third sensitizing treatment, 0.2 ml of a 50% aqueous dilution of Freund's adjuvant[2] per animal was injected intradermally using two or three sites next to the test site. Following the fourth sensitizing dose, the animals were rested for two weeks. Both flanks were then clipped and challenged on one flank with the test material and the other flank with the vehicle. The challenge application was not occluded. The skin response at these sites was recorded at 24 and 48 h after application according to the evaluation method provided in Appendix 4. Any animal eliciting a score of two or more at the test solution challenge site at the 48-h scoring was rated a positive responder. The frequency of the reaction is the important statistic in determining sensitization potential. Appendix 5 was used to classify the test materials as to sensitization potential.

INHALATION TOXICITY

Aerosol Generation System

The aerosol generation system consisted of a large polyvinylchloride generation vessel containing five 6-jet Collison[3] compressed air nebulizers (Figure 3). The nebulizers were installed within the generation vessel in a manner that allowed an equidistant impaction surface for each. Test material in the generation vessel was maintained at a constant by a recycling pump regulated to supply test material at a rate equivalent to generator consumption.

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1. Surgex Hair Remover Cream, Sparta Instrument Corporation, Hayward, CA
2. Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, MI
3. BGI, Inc., Waltham, MA 02154

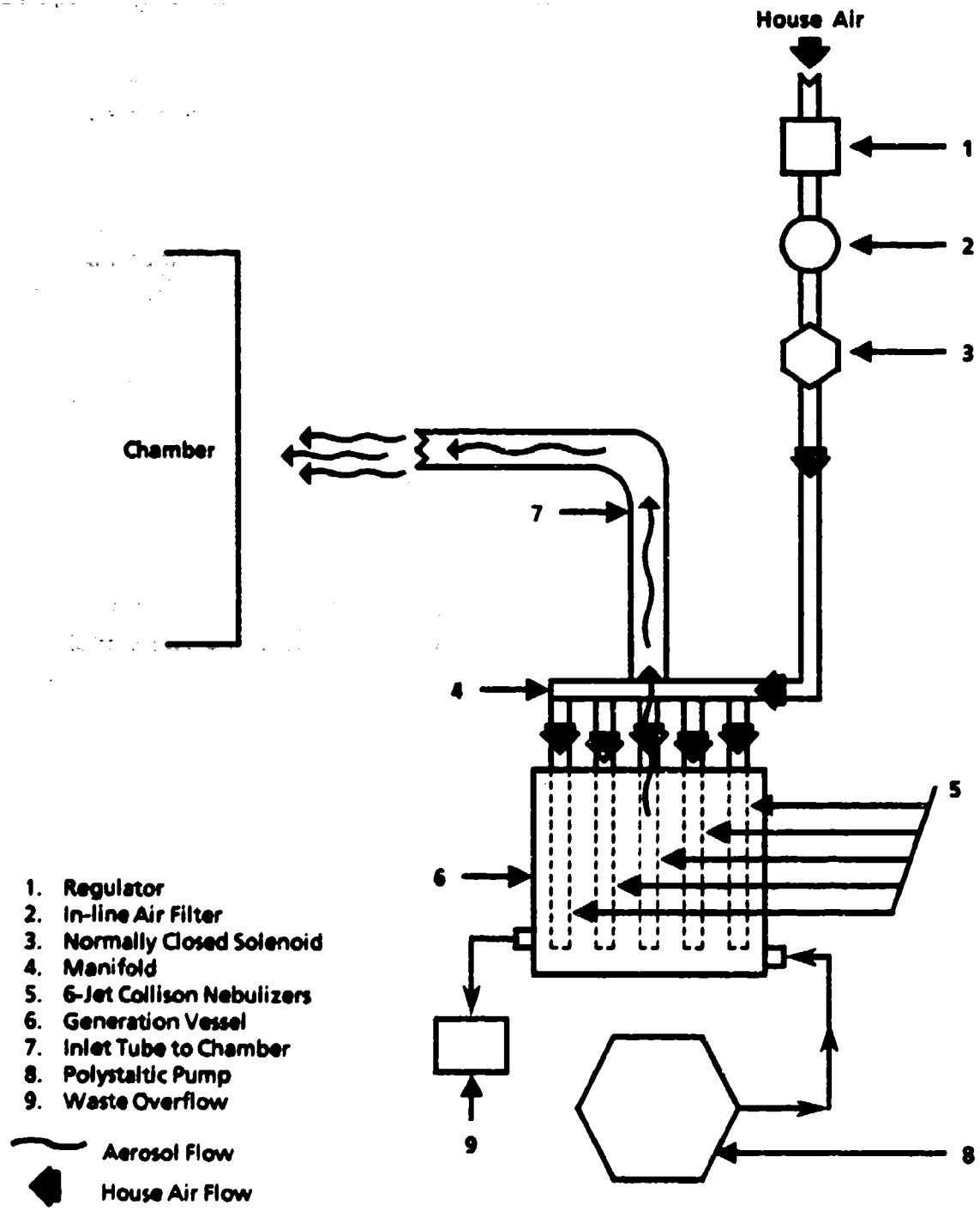

Figure 3. Generation system used for inhalation studies.

Analysis of Chamber Atmospheres

Seven Metricel[4] membrane filter samples of chamber atmospheres were taken during the course of each 4-h exposure. These were gravimetrically analyzed for mass concentration by dividing the weight change by the sample volume. These same filters were used to determine nonvolatile concentrations, as described below.

Aerosol particle size distributions were measured with a Lovelace Multijet Impactor[5]. One 10-min sample was taken for each exposure. The vapor phase of the chamber atmosphere was analyzed for ethylene glycol concentration using a Miran 1A at a 9.5-μm wavelength. The percent nonvolatile materials in the fluids was determined by collecting aerosol on a preweighed (Wt. 1) Metricel filter (the same filter samples used to determine gravimetric measurements). The samples were reweighed (Wt. 2), heated in a drying oven for 10 min at 80°C, then cooled and weighed again (Wt. 3) to determine weight loss. Blank filters were treated in the same manner and the average loss added to the final weight. The percent nonvolatiles was determined using the following equation:

$$\frac{\text{Wt. 3 - Wt. 1 + Control Wt. Loss}}{\text{Wt. 2 - Wt. 1}} \times 100$$

Exposure Regimen

Five male and five female F-344 rats, age 10 weeks, were placed in a 2.22-m^3 Rochester chamber with an effective volume of 1.45 m^3 and exposed for 4 h to the maximum achievable concentration of aerosolized test material. The limit test concentration of 5000 mg/m^3 could not be produced because of the physical properties of the test materials. Records were maintained for body weights (day 0, 7, 10, and 14 post-exposure), signs of toxicity, and mortality. Gross pathology was performed on the day of sacrifice.

STATISTICAL ANALYSIS

Body weight mean ± standard error of the mean (S.E.M.) was calculated according to Dixon (1985). Comparison of body weights was performed using the Multivariate Analysis of Covariance for Repeated Measures Test (Barcikowski, 1983; Dixon, 1985). A significant change from controls was inferred when the probability was 0.05 or less.

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4. Gelman Sciences, Inc., Ann Arbor, MI 48106
5. Intox Products, Albuquerque, NM 87110

SECTION 3

RESULTS

ORAL TOXICITY

A total of 40 F-344 rats (five male and five female per test material) were orally dosed. During the 14-day observation period, all animals gained weight (Figures 4 through 7 and Appendix 6) and showed no clinical signs of toxicity. A 50.0, 49.9, 49.9, 47.6, and 45.3% gain in body weight was shown in the male Control, 6049-1, 6049-2, 6049-3, and 6049-4 animals, respectively. A 29.5, 27.7, 30.2, 28.7, and 29.4% gain in body weight was shown in the female Control, 6049-1, 6049-2, 6049-3, and 6049-4 rats, respectively. Body weights on day 0 (first day of study) and on day 14 (final day of study) did not differ significantly ($p < 0.05$) between the control animals and the treatment groups using the Multivariate Analysis of Covariance for Repeated Measures Test (Barcikowski, 1983).

On the 14th day post-exposure, gross pathology was performed and select gross lesions were harvested for microscopic examination. These tissues failed to reveal any treatment-related changes.

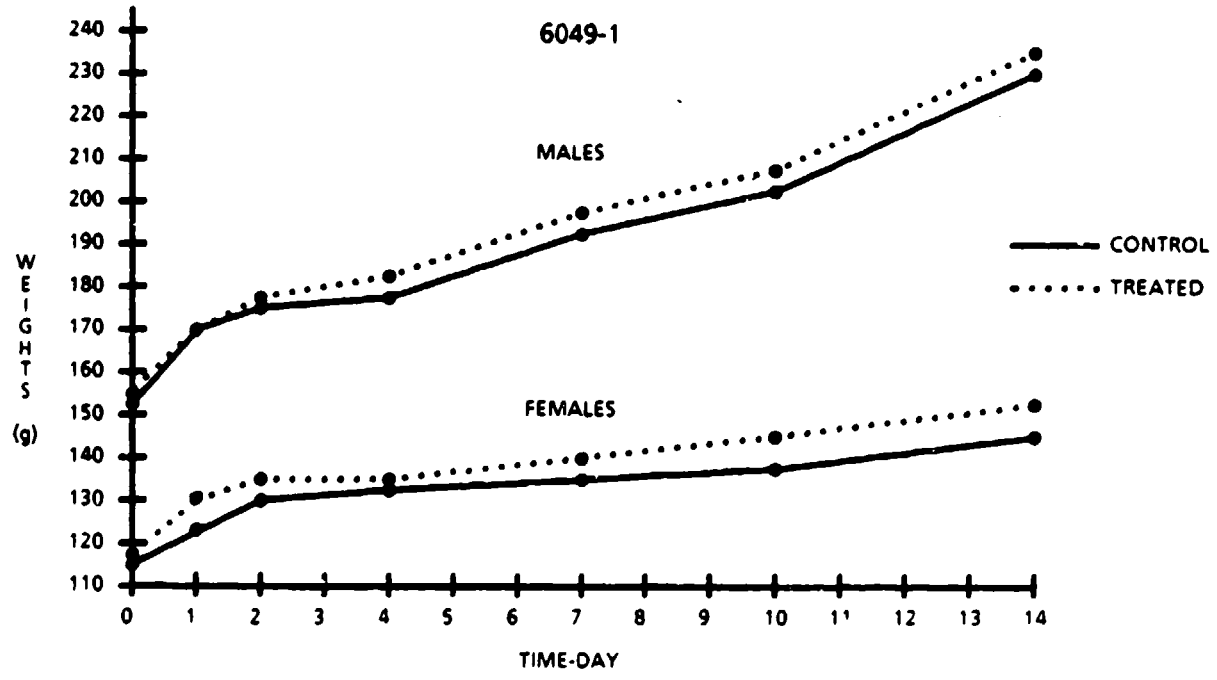

Figure 4. Body weights for test and control male and female F-344 rats orally dosed with test compound 6049-1.

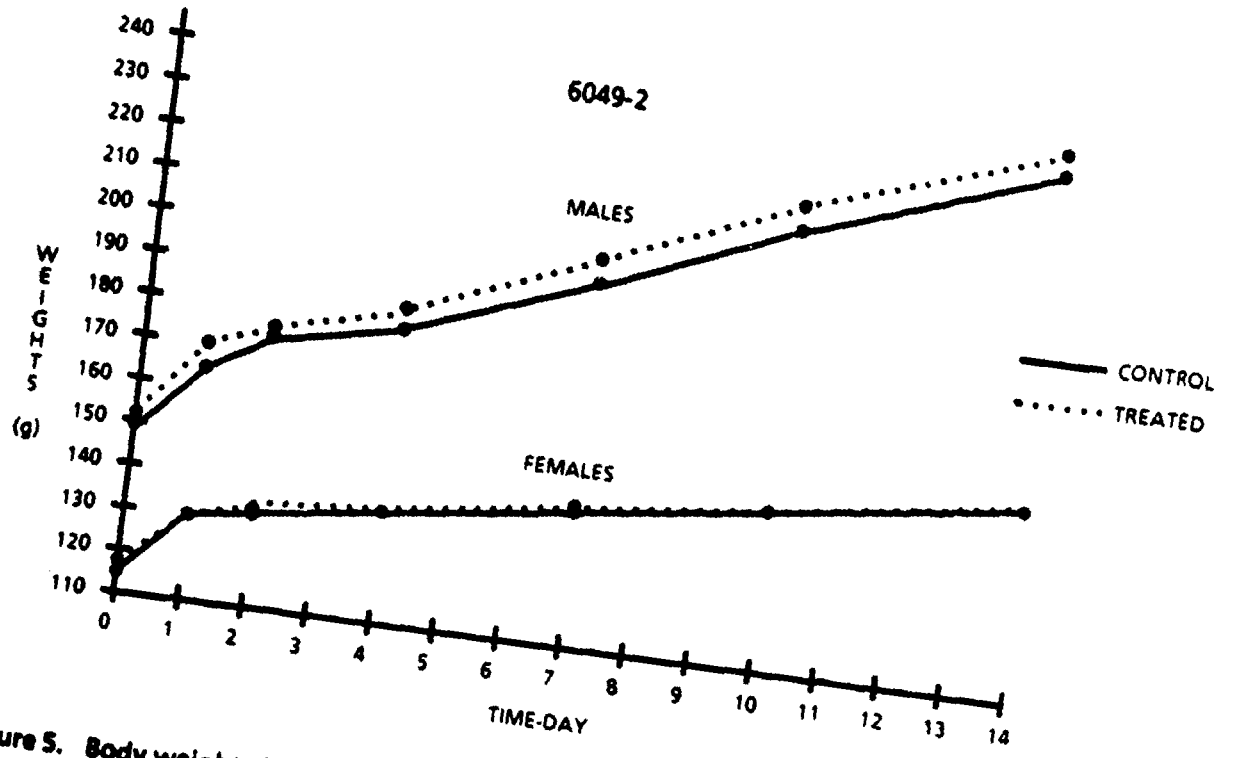

Figure 5. Body weights for test and control male and female F-344 rats orally dosed with test compound 6049-2.

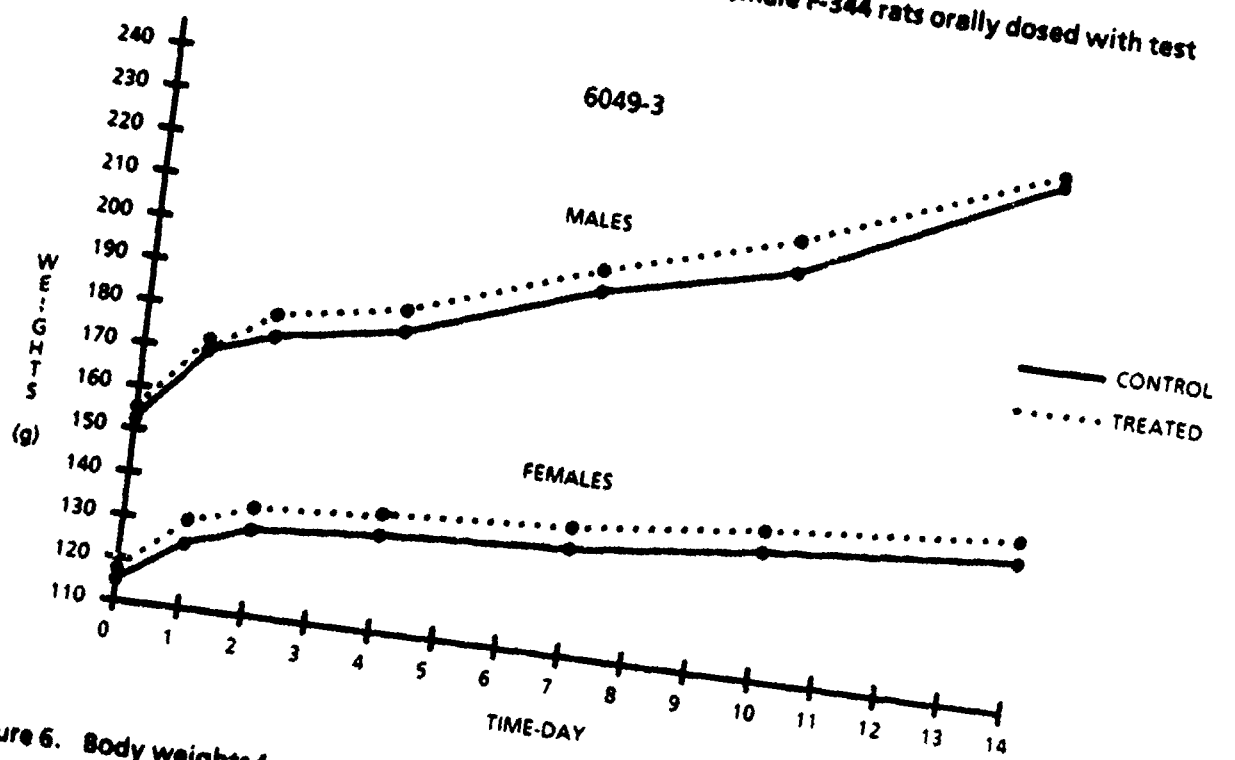

Figure 6. Body weights for test and control male and female F-344 rats orally dosed with test compound 6049-3.

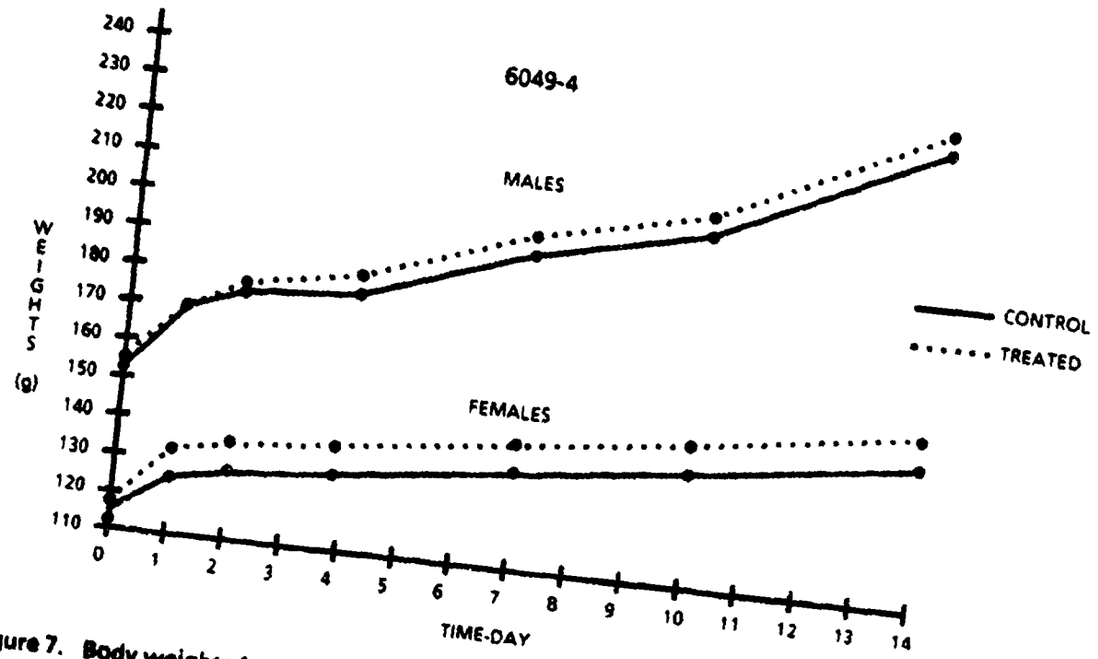

Figure 7. Body weights for test and control male and female F-344 rats orally dosed with test compound 6049-4.

DERMAL TOXICITY

Ten New Zealand white rabbits (five male, five female) were used per treatment group. No deaths resulted from the 24-h skin contact with the four test materials at a dose of 2 g/kg. All animals gained weight (Appendix 7) and showed no toxic signs over the 14-day post-treatment observation period. The percent body weight gain was 11.3, 8.9, 8.9, and 9.6 for the males in treatment groups 6049-1, 6049-2, 6049-3, and 6049-4, respectively. For the females, the percent body weight gain was 10.9, 12.7, 10.8, and 14.7 for treatment groups 6049-1, 6049-2, 6049-3, and 6049-4, respectively.

EYE IRRITATION

Thirty-six animals were used (nine per test substance) in this facet of the study. In prescreening the rabbit eyes with fluorescein stain prior to treatment, several showed mild to intense staining of the corneal epithelium. The eyes showing opacity were not used in the study, however, all through the study the eyes of the rabbits would show opacity on one evaluation and would be clear on another screening.

Mild conjunctival redness was present in two rabbits 1 h after treatment with 6049-1. However, no redness was present at 24, 48, or 72 h. Six of the nine animals treated with 6049-2 showed mild conjunctival redness at the 1-h screening, and two of the six showed redness at the 72-h evaluation. Seven of the nine animals treated with 6049-3 showed mild conjunctival redness at the 1-h screening, and one had persistent redness at the 72-h evaluation. Of the nine animals treated with 6049-4, five showed mild conjunctival redness at the 1-h evaluation; no redness persisted at the 72-h screening (Appendix 9).

None of the animals showed any inflammation or irritation of the iris.

SKIN IRRITATION

Following 4 h of skin contact, none of the rabbits from any treatment group showed signs of necrosis or edema. One of the six rabbits dosed with test compound 6049-1 showed signs of erythema 4 h post-exposure. Twenty-four hours after treatment, three of the six rabbits tested with this material showed signs of erythema and one showed signs of erythema and edema. At 48 h post-exposure, three continued to show erythema, while at 72 h, two showed erythema. Of the rabbits treated with compound 6049-2, one showed signs of erythema at the 24-h observation, while no others showed any irritating signs at the other observation periods. No rabbits dosed with compound 6049-3 showed signs of erythema or edema 4 h post-treatment; however, one rabbit displayed erythema at the 24-, 48-, and 72-h evaluation. Following treatment with compound 6049-4, one rabbit exhibited erythema at 4 h, four rabbits at 24 h, one at 48 h, and two at the 72-h screening (see Appendix 10). Skin reactions were evaluated and scored (Table 2) according to Draize (Appendix 2) and interpreted according to NIOSH Interpretation of Skin Test Ratings (Appendix 3). These ratings revealed all four water-in-oil test compounds to be non-irritating.

TABLE 2. SUMMARY OF RABBIT SKIN IRRITATION EFFECTS AFTER 4-HOUR CONTACT WITH FOUR WATER-IN-OIL EMULSIONS

Compound	Time Post-exposure			
	4-h	24-h	48-h	72-h
	Erythema			
6049-1	Very Slight (1)	Very Slight (4)	Very Slight (3)	Very Slight (2)
6049-2	Very Slight (0)	Very Slight (1)	Very Slight (0)	Very Slight (0)
6049-3	Very Slight (0)	Very Slight (1)	Very Slight (1)	Very Slight (1)
6049-4	Well Defined (1)	Very Slight (4)	Very Slight (1)	Very Slight (2)

The number in ( ) indicates the number of rabbits exhibiting signs out of a possible six animals per treatment group.

The parameters of (1) erythema, (2) edema, and (3) necrosis were evaluated for each chemical on all animals using the scoring system of Draize (Appendix 2). The total score of the four observations for all rabbits was divided by 24 to yield a primary irritation rating (Table 3), which was interpreted using the NIOSH skin test rating.

TABLE 3. RABBIT PRIMARY SKIN IRRITATION RESULTS

Test Compound	Primary Irritation Index	Effect
6049-1	0.46	Non-irritant
6049-2	0.04	Non-irritant
6049-3	0.13	Non-irritant
6049-4	0.38	Non-irritant

SENSITIZATION

Ten guinea pigs per test compound were treated with a 10% dilution of the compound in mineral oil during the challenge application. A weak sensitization reaction was shown with test substance 6049-1. No animals treated with the challenge application of the other three test substances showed a sensitization response (Appendix 11). Any guinea pig with a score of two or above was considered sensitized. The grading scale for these scores is presented in Appendices 4 and 5.

INHALATION TOXICITY

The chemical analysis of the water-in-oil emulsion aerosols from the exposure atmospheres showed that the amount of ethylene glycol (the most toxic substance thought was present in the test materials) was well within the acceptable standard of 125 mg/m^3 ceiling concentration (ACGIH, 1985). These data as well as the data on mass median aerodynamic diameter (MMAD), mean aerosol concentration and percent nonvolatiles present are shown in Table 4.

Ten F-344 rats (five male and five female) were used per test compound. No deaths occurred as a result of any of the inhalation exposures. All animals gained weight over the two-week post-exposure period (Figures 8 through 11 and Appendix 8) and showed no signs of toxicity. There was no statistical difference ($p < 0.05$) in percent body weight gain between the controls and their corresponding treatment groups (males or females) for test compounds 6049-2, 6049-3, or 6049-4. Percent body weight gain for rats (male and female) exposed to 6049-1 showed a significant statistical difference ($p < 0.05$) between the treatment group and its corresponding control group.

On the day of sacrifice, select gross lesions were harvested for microscopic examination. These lesions failed to reveal significant target organ toxicity or any treatment-related changes.

The respirable range for the aerodynamic diameter of an aerosol is 0.20 - 5.0 micrometers (ACGIH, 1985). The aerosols of all four water-in-oil emulsions fell well within the respirable range.

TABLE 4. CHEMICAL ANALYSES OF FOUR WATER-IN-OIL EMULSIONS DURING 4-HOUR INHALATION

Test Compound	Mean Concentration (mg/m^3)	MMAD[a] (µm) ± S.G.D.[b]	Ethylene Glycol (mg/m^3)	Nonvolatiles (%)
6049-1	180	2.00 ± 1.90	37	84
6049-2	110	2.25 ± 1.78	29	79
6049-3	210	2.15 ± 1.77	23	87
6049-4	180	1.90 ± 2.00	11	75

[a]Mass median aerodynamic diameter

[b]Standard geometric deviation

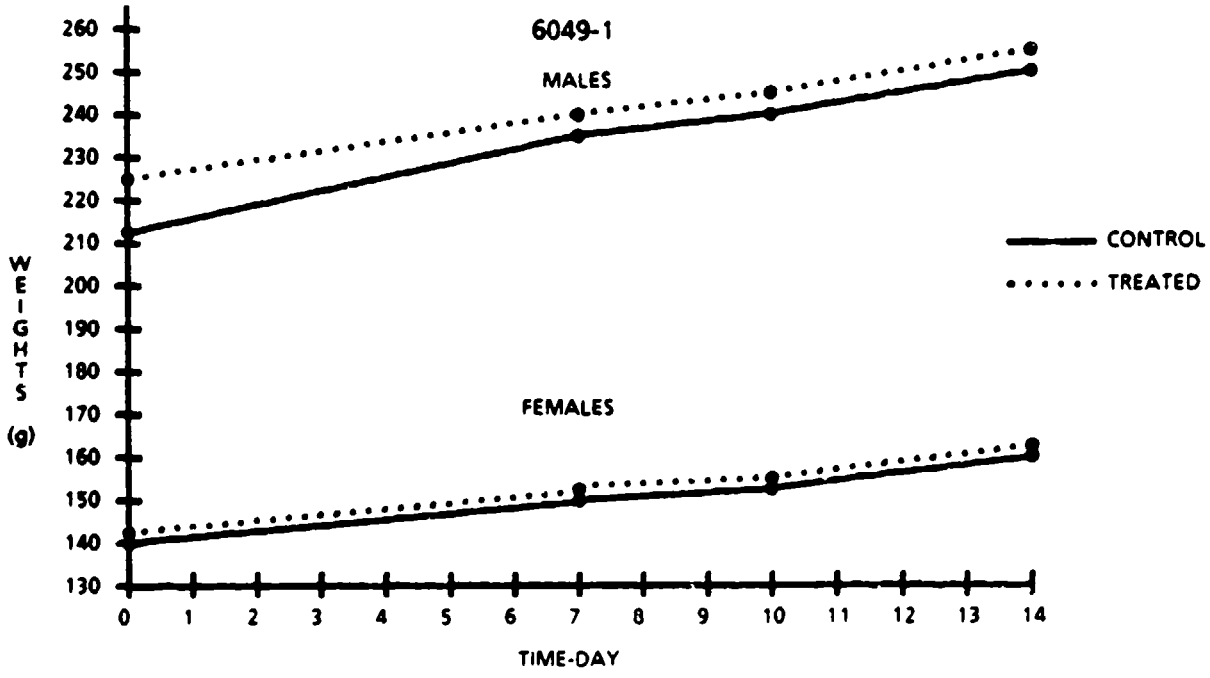

Figure 8. **Body weights for test and control male and female F-344 rats exposed to test compound 6049-1 during inhalation studies.**

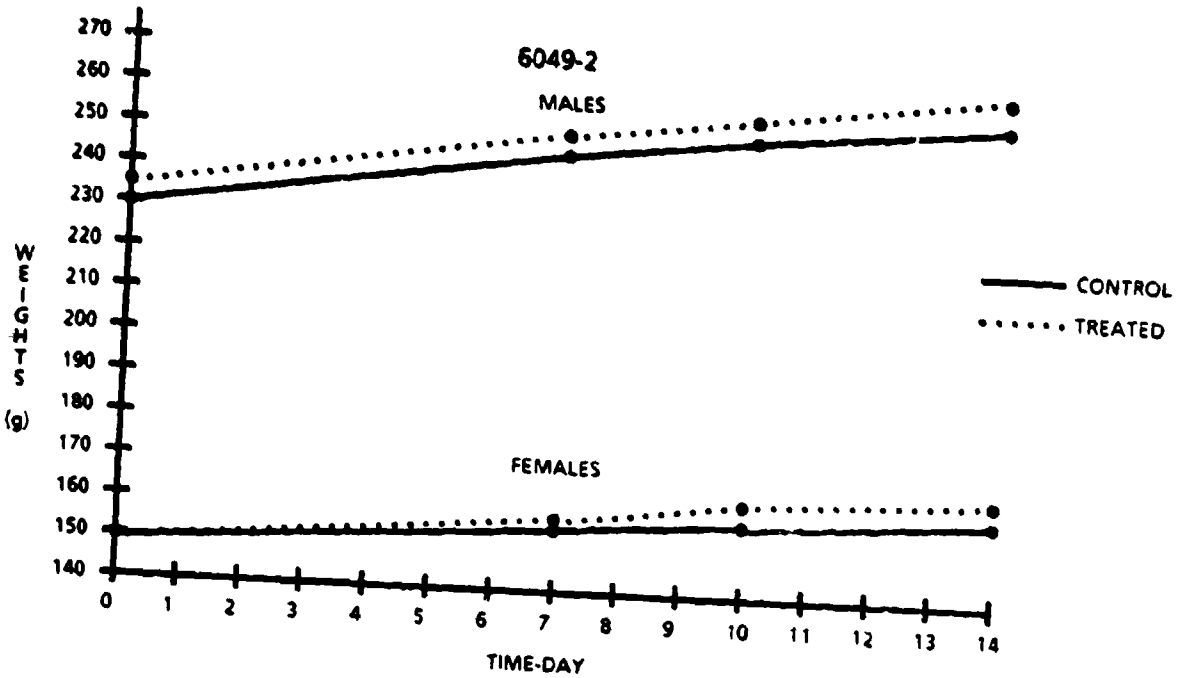

Figure 9. Body weights for test and control male and female F-344 rats exposed to test compound 6049-2 during inhalation studies.

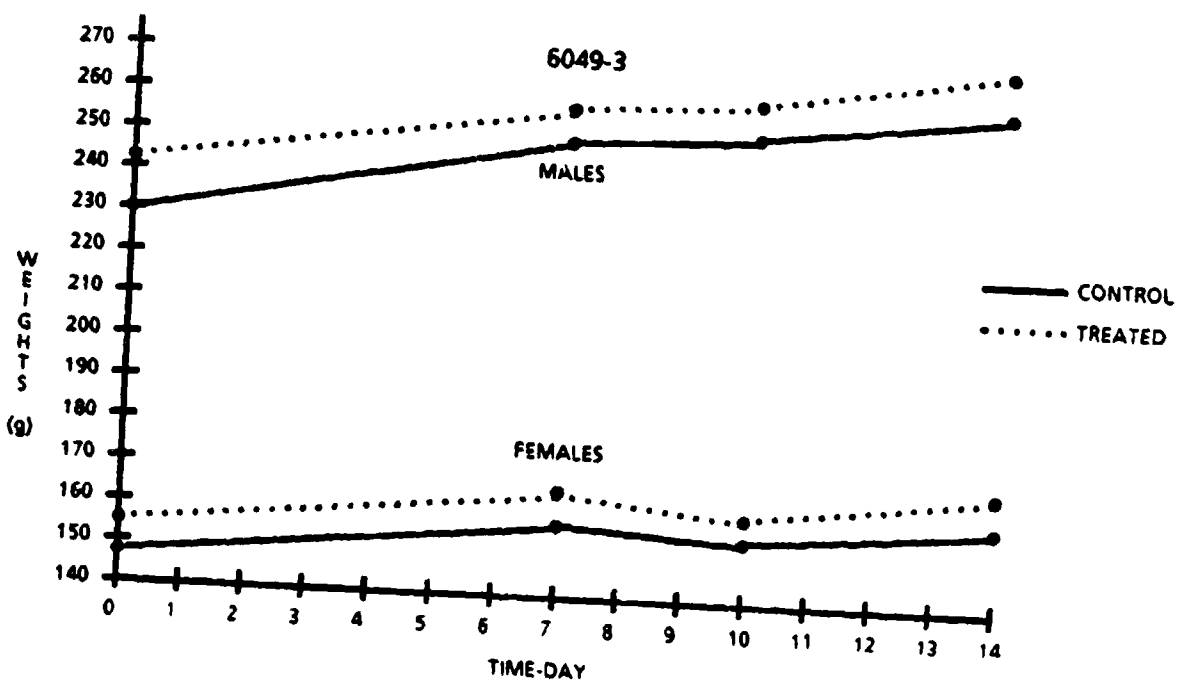

Figure 10. Body weights for test and control male and female F-344 rats exposed to test compound 6049-3 during inhalation studies.

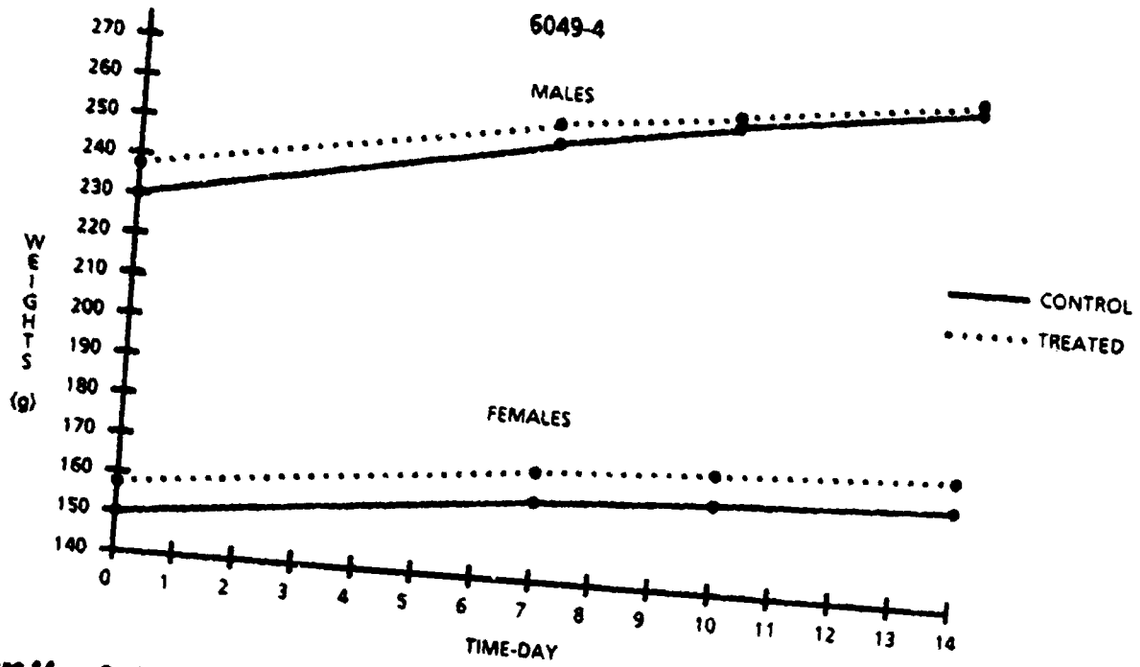

Figure 11. **Body weights for test and control male and female F-344 rats exposed to test compound 6049-4 during inhalation studies.**

SECTION 4

DISCUSSION

In the oral, dermal, and inhalation toxicity studies, no deaths or toxic signs were observed with any of the four test compounds. During necropsy, select gross lesions were harvested from 13 rats in the oral and inhalation studies. Microscopic examination of these lesions failed to reveal significant target organ toxicity or any other treatment-related changes. Generally, all lesions were regarded as incidental and within normal limits of variation for rat organs. All four compounds exhibited a mild irritating effect to the conjunctival tissue of rabbit eyes. Additionally, all through the study the eyes of the rabbits would show opacity on one evaluation and would be clear on another screening. Kikkawa (1972) noted a similar phenomenon and hypothesized that this was a result of normal desquamation of the corneal epithelium and not due to instillation of the test compounds. Inconsistencies in our observations tend to support that conclusion. Significant irritating effects were not observed with any of the four test materials as a result of exposure to intact skin of rabbits.

The skin sensitization test was designed to evaluate the potential of materials to act as antigens. Applications of small quantities of antigenic material over a period of time induces

antibody production. The induction potential can then be evaluated by grading the response to a challenge administration of the material. The 48-h response of one guinea pig to the 6049-1 test substance indicated the possibility of the material being a weak sensitizer. The remaining test compounds did not elicit a sensitization reaction.

Table 5 is a summary of the test results of all studies conducted under this technical directive.

TABLE 5. COMPARISON OF ACUTE TEST RESULTS OF THE FOUR WATER-IN-OIL EMULSION FLUIDS

Test Compound	Oral LD_{50} (g/kg)	Dermal LD_{50} (g/kg)	Inhalation LC_{50} (mg/m^3)	Eye Irritation	Skin Irritation	Sensitization Irritation
6049-1	>5[a]	>2[a]	>180[a]	mild	non-irritant (0.46)[b]	weak[c]
6049-2	>5[a]	>2[a]	>110[a]	mild	non-irritant (0.04)[b]	negative[c]
6049-3	>5[a]	>2[a]	>210[a]	mild	non-irritant (0.13)[b]	negative[c]
6049-4	>5[a]	>2[a]	>180[a]	mild	non-irritant (0.38)[b]	negative[c]

[a] No deaths or toxic signs at these concentrations

[b] Based on NIOSH Skin Test Rating (0-0.9 = non-irritant)

[c] Tested as 10% solution in mineral oil

SECTION 5

REFERENCES

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Dixon, W. J. 1985. BMDP Statistical Software. Berkeley, CA: University of California Press.

Draize, J. H. 1959. Dermal Toxicity. In: The Staff of the Division of Pharmacology of the Federal Food and Drug Administration, Appraisal of the Safety of Chemicals in Food, Drugs, and Cosmetics, pp. 46-59. Austin, TX: The Editorial Committee of the Associates of Food and Drug Officials of the United States.

Draize, J. H., G. Woodard, and H. O. Calvery. 1944. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharm. Exp. Therap.* 32:377-390.

Kikkawa, Y. 1972. Normal Corneal Staining with Fluorescein. *Exp. Eye Res.* 14:13-20.

The American Conference of Governmental Industrial Hygienists. 1985. Threshold Limit Values and Biological Exposure Indices for 1985-1986. (ISBN: 0-936712-61-9) Cincinnati, OH.

SECTION 6

QUALITY ASSURANCE

The study "The Evaluation of the Acute Toxicity of Four Water-in-Oil Emulsion Hydraulic Fluids" was conducted by Northrop Services, Inc. – Environmental Sciences, Toxic Hazards Research Unit to be in compliance with the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. The various phases of this study were inspected by members of the Quality Assurance Group. Results of these inspections were reported directly to the Technical Manager (Study Director) at the close of each inspection.

Date of Inspection	Item Inspected
July 29, 1986	Dermal LD_{50} Limit Test
October 8, 1986	Data Audit
June 17, 1987	Report Audit
June 26, 1987	Data Audit
June 30, 1987	Notebook Audit
July 21, 1987	Data Audit

The Quality Assurance Group has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study.

M.G. Schneider
QA Coordinator
Northrop Services, Inc.
Environmental Sciences
Toxic Hazards Research Unit

Date September 2, 1987

APPENDIX 1

DRAIZE[1] SCALE FOR SCORING OCULAR LESIONS

Parameter	Score
1. CORNEA	
A. Opacity-degree of density (area most dense taken for reading)	
No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One-quarter (or less), but not zero	1
Greater than one-quarter, but less than one-half	2
Greater than one-half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4
Score = A x B x 5	Total Maximum = 80
2. IRIS	
A. Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof); iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
Score equals A x 5	Total Maximum = 10
3. CONJUCTIVAE	
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

APPENDIX 1 (continued)

DRAIZE[1] SCALE FOR SCORING OCULAR LESIONS

B. Chemosis	
No swelling	0
Any swelling above normal (included nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids from more than half closed to completely closed	4
C. Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Score equals (A + B + C) x 2	Total Maximum = 20

The MAXIMUM TOTAL SCORE is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Total maximum score possible =	110

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[1]Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharm. Exp. Therap.* 32:377-390.

APPENDIX 2

DRAIZE[1] SCALE FOR EVALUATION AND SCORING OF SKIN REACTIONS

Parameter	Score
1. Erythema	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4
2. Edema	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raising approx. 1 mm)	3
Severe edema (raising more than 1 mm and extending beyond area of exposure)	4
3. Necrosis[2]	
No necrosis	0
Slight necrosis (less than one-fourth exposed area)	5
Moderate necrosis (one-fourth to one-half exposed area)	10
Severe necrosis (more than one-half exposed area)	15

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[1] Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharm. Exp. Therap.* 32:377-390.

[2] Necrosis, for the purpose of this scoring system, is defined as a chemical denaturation of tissue sufficiently severe to result in fibrotic replacement (scar tissue). Superficial eschar that heals without scar is not classified as necrosis.

APPENDIX 3

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH INTERPRETATION OF SKIN TEST RATINGS[1]

	Rating	Interpretation
Intact skin	0-0.9	Non-irritant; probably safe for intact human skin contact
	1-1.9	Mild irritant; may be safe for use, but appropriate protective measures are recommended during contact
	2-4	Too irritating for human skin contact; avoid contact

[1] Campbell, K.I., E.L. George, L.L. Hale, and J.F. Stara. 1975. Dermal Irritancy of Metal Compounds. *Arch. Environ. Health.* 30:168-170.

APPENDIX 4

GRADING SYSTEM[1] FOR SENSITIZATION TEST

ERYTHEMA	EDEMA
0 - None	0 - None
1 - Very Slight Pink	1 - Very Slight
2 - Slight Pink	2 - Slight
3 - Moderate Red	3 - Moderate
4 - Very Red	4 - Marked

[1] Toxic Hazards Research Unit grading system for sensitization test.

APPENDIX 5

SCALE[1] FOR DETERMINING SENSITIZATION POTENTIAL

Sensitization Rate (%)	Grade
10	Weak
20-30	Mild
40-60	Moderate
70-80	Strong
90-100	Extreme

[1] Toxic Hazards Research Unit scale for determining sensitization potential.

APPENDIX 6

BODY WEIGHTS (g) OF F-344 RATS IN ORAL TOXICITY STUDY

CONTROL ANIMALS

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
0070001	144	161	165	174	188	195	210
0070003	144	165	168	176	192	204	223
0070011	162	180	186	195	211	222	238
0070019	155	174	179	186	202	213	232
0070021	151	171	175	183	200	210	231
Mean	151	170	175	183	199	209	227
(± S.E.M.)	(3.4)	(3.3)	(3.8)	(3.8)	(4.0)	(4.5)	(4.8)
Females							
0070037	124	137	140	142	147	151	154
0070039	118	131	133	135	140	143	152
0070040	103	116	118	120	128	132	138
0070046	119	132	137	137	144	150	158
0070049	123	136	138	142	147	151	158
Mean	117	130	133	135	141	145	152
(± S.E.M.)	(3.8)	(3.8)	(4.0)	(4.0)	(3.5)	(3.7)	(3.7)

TREATMENT GROUP 6049-1

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
0070008	140	153	160	168	185	200	217
0070009	159	175	182	193	207	221	238
0070012	157	173	177	187	202	217	228
0070018	147	163	170	178	195	212	228
0070022	173	191	193	205	220	236	252
Mean	155	171	176	186	202	217	233
(± S.E.M.)	(5.6)	(6.4)	(5.6)	(6.3)	(5.9)	(5.9)	(5.9)
Females							
0070032	109	121	125	129	137	138	147
0070043	108	115	120	126	132	134	143
0070044	125	132	137	143	149	150	159
0070051	125	134	138	142	143	147	150
0070052	107	116	120	122	124	127	134
Mean	115	124	128	132	137	139	147
(±S.E.M.)	(4.2)	(4.0)	(4.0)	(4.3)	(4.3)	(4.2)	(4.1)

TREATMENT GROUP 6049-2

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
0070002	157	173	178	185	194	202	220
0070004	146	158	169	178	192	203	223
0070017	150	168	173	185	201	211	230
0070023	153	169	178	186	205	215	231
0070026	138	153	160	166	183	195	211
Mean	149	164	172	180	195	205	223
(± S.E.M.)	(3.3)	(3.7)	(3.4)	(3.8)	(3.8)	(3.5)	(3.7)
Females							
0070028	111	124	127	128	134	139	144
0070035	122	133	135	140	144	150	156
0070042	122	136	139	142	147	153	159
0070050	114	127	130	134	138	141	147
0070054	114	128	128	132	139	143	153
Mean	117	130	132	135	140	145	152
(± S.E.M.)	(2.3)	(2.2)	(2.3)	(2.6)	(2.3)	(2.7)	(2.8)

TREATMENT GROUP 6049-3

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
0070013	160	176	182	194	210	222	232
0070016	159	177	182	192	207	220	231
0070020	155	169	178	185	202	215	229
0070025	149	165	172	181	196	207	226
0070032	153	170	177	184	199	212	227
Mean	155	171	178	187	203	215	229
(± S.E.M.)	(2.0)	(2.3)	(1.9)	(2.5)	(2.6)	(2.7)	(1.1)
Females							
0070029	120	133	135	137	143	148	157
0070030	115	127	130	132	138	140	146
0070036	111	118	124	127	134	140	148
0070038	114	127	130	134	138	140	144
0070045	119	133	136	138	144	147	150
Mean	116	128	131	134	139	143	149
(± S.E.M.)	(1.7)	(2.8)	(2.1)	(2.0)	(1.8)	(1.8)	(2.2)

TREATMENT GROUP 6049-4

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
0070005	165	184	190	199	215	228	240
0070006	167	184	190	202	218	232	249
0070007	167	185	190	200	215	230	248
0070010	150	167	175	184	198	212	225
0070014	163	179	180	183	193	205	218
Mean	162	180	185	194	208	221	236
(± S.E.M.)	(3.2)	(3.4)	(3.2)	(4.2)	(5.1)	(5.4)	(6.2)
Females							
0070031	114	126	130	134	138	145	153
0070033	112	122	125	127	133	136	142
0070034	118	128	132	135	141	143	150
0070048	108	119	123	125	129	132	139
0070053	113	125	128	129	136	140	147
Mean	113	124	128	130	135	139	146
(± S.E.M.)	(1.6)	(1.6)	(1.6)	(2.0)	(2.1)	(2.4)	(2.6)

APPENDIX 7

BODY WEIGHTS (kg) OF MALE AND FEMALE RABBITS AFTER 24-HOUR DERMAL EXPOSURE

TREATMENT GROUP 6049-1

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
V04	2.96	2.89	2.88	3.00	3.03	3.14	3.17
V14	3.05	2.98	3.07	3.20	3.18	3.30	3.33
V26	2.60	2.60	2.71	2.95	2.88	2.95	2.98
V30	2.70	2.65	2.60	2.87	2.87	2.96	3.04
V38	2.98	2.88	2.96	3.50	3.21	3.30	3.37
Mean	2.86	2.80	2.84	3.10	3.03	3.13	3.18
(±S.E.M.)	0.09	0.07	0.08	0.11	0.07	0.08	0.08
Females							
P65	2.92	2.75	2.83	2.95	3.05	3.22	3.28
P73	2.75	2.68	2.72	2.84	2.79	2.87	2.88
P83	2.70	2.62	2.69	2.80	2.84	2.99	2.92
P85	2.91	2.78	2.90	3.00	3.05	3.20	3.37
P89	2.90	2.71	2.79	3.00	3.00	3.18	3.29
Mean	2.84	2.71	2.79	2.92	2.95	3.09	3.15
(±S.E.M.)	0.05	0.03	0.04	0.04	0.05	0.07	0.10

TREATMENT GROUP 6049-2

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
V02	2.82	2.75	2.80	2.95	2.90	3.05	3.11
V18	2.78	2.78	2.87	3.20	3.05	3.15	3.23
V22	2.57	2.52	2.53	2.71	2.68	2.72	2.73
V32	2.85	2.75	2.77	2.95	2.82	2.94	2.96
V34	2.83	2.82	2.82	2.91	2.91	3.00	3.05
Mean	2.77	2.72	2.76	2.94	2.87	2.97	3.02
(±S.E.M.)	0.05	0.05	0.06	0.08	0.06	0.07	0.08
Females							
P69	3.05	2.88	3.00	3.20	3.18	3.39	3.48
P71	2.86	2.89	2.99	3.50	3.17	3.08	3.26
P81	2.85	2.75	2.83	3.00	3.04	3.10	3.23
P87	2.70	2.68	2.70	2.85	2.93	3.04	3.10
P91	2.70	2.58	2.65	2.82	2.70	2.89	2.90
Mean	2.83	2.76	2.83	3.07	3.00	3.10	3.19
(±S.E.M.)	0.06	0.06	0.07	0.13	0.09	0.08	0.10

TREATMENT GROUP 6049-3

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
V08	2.75	2.75	2.64	2.67	2.65	2.77	2.84
V12	3.14	2.92	3.05	3.50	3.21	3.25	3.40
V16	2.88	2.78	2.84	2.92	3.10	3.19	3.29
V20	3.10	3.08	3.04	3.05	3.21	3.28	3.33
V24	2.98	2.88	2.92	3.01	3.13	3.22	3.32
Mean	2.97	2.88	2.90	3.03	3.06	3.14	3.24
(±S.E.M.)	0.07	0.06	0.08	0.13	0.10	0.09	0.10
Females							
P63	2.90	2.82	2.90	2.97	3.05	3.18	3.34
P67	2.75	2.69	2.71	2.69	2.64	2.83	2.81
P75	2.90	2.77	2.83	2.92	3.09	3.03	3.20
P77	2.89	2.80	2.87	2.86	2.98	3.14	3.27
P97	2.87	2.78	2.88	3.00	3.08	3.16	3.24
Mean	2.86	2.77	2.84	2.89	2.97	3.07	3.17
(±S.E.M.)	0.03	0.02	0.03	0.05	0.08	0.06	0.09

TREATMENT GROUP 6049-4

Animal No.	Day 0	Day 1	Day 2	Day 4	Day 7	Day 10	Day 14
Males							
V06	2.85	2.80	2.80	2.80	2.88	3.02	3.08
V10	2.74	2.60	2.63	2.67	2.83	2.93	2.98
V28	2.65	2.68	2.64	2.70	2.72	2.80	2.88
V36	3.04	2.94	3.02	3.01	3.12	3.18	3.31
V40	2.85	2.89	2.89	2.92	3.10	3.20	3.23
Mean	2.83	2.78	2.80	2.82	2.93	3.03	3.10
(±S.E.M.)	0.06	0.06	0.07	0.06	0.08	0.08	0.08
Females							
P79	2.70	2.60	2.58	2.70	2.82	2.92	3.00
P93	2.81	2.79	2.80	2.92	3.00	3.10	3.14
P95	2.72	2.70	2.72	2.80	2.90	3.00	3.09
P99	2.88	2.90	2.93	3.00	3.21	3.36	3.47
Q01	2.83	2.78	2.84	3.00	3.05	3.13	3.30
Mean	2.79	2.75	2.77	2.88	3.00	3.10	3.20
(±S.E.M.)	0.03	0.05	0.06	0.06	0.07	0.07	0.08

APPENDIX 8

BODY WEIGHTS (g) OF MALE AND FEMALE RATS AFTER 4-HOUR INHALATION EXPOSURE

TREATMENT GROUP 6049-1

Animal No.	Day 0	Day 7	Day 10	Day 14
CONTROLS				
Males				
0070127	214	244	246	254
0070134	195	223	223	237
0070138	219	246	247	257
0070143	213	240	244	256
0070147	216	242	248	256
Mean	211	239	242	252
(±S.E.M.)	4.2	4.1	4.7	3.8
Females				
0070154	145	158	157	164
0070156	142	157	160	165
0070160	135	146	152	154
0070175	138	153	154	157
0070176	139	148	149	155
Mean	140	152	154	159
(±S.E.M.)	1.7	2.4	1.9	2.3
TREATED				
Males				
0070120	226	246	248	258
0070130	239	260	265	275
0070133	215	235	236	246
0070144	227	250	254	262
0070145	206	225	229	239
Mean	223	243	246	256
(±S.E.M.)	5.6	6.1	6.4	6.3
Females				
0070148	146	160	163	168
0070151	144	158	158	164
0070162	137	152	150	157
0070166	143	155	157	161
0070173	143	151	155	157
Mean	143	155	157	161
(±S.E.M.)	1.5	1.7	2.1	2.1

TREATMENT GROUP 6049-2

Animal No.	Day 0	Day 7	Day 10	Day 14
CONTROLS				
Males				
0070127	232	253	254	262
0070134	216	231	237	239
0070138	237	254	257	266
0070143	232	250	256	261
0070147	235	253	256	260
Mean	230	248	252	258
(±S.E.M.)	3 7	4.4	3.8	4.8
Females				
0070154	154	161	164	163
0070156	152	165	165	163
0070160	144	151	154	157
0070175	147	157	157	157
0070176	148	150	155	156
Mean	149	157	159	159
(±S.E.M.)	1.8	2.9	2.3	1.6
TREATED				
Males				
0070124	222	243	247	254
0070128	232	255	259	265
0070135	236	252	256	257
0070137	239	258	261	270
0070142	240	262	269	280
Mean	234	254	258	265
(±S.E.M.)	3.3	3.2	3.6	4.7
Females				
0070155	142	147	153	151
0070158	150	160	161	163
0070164	146	158	163	162
0070167	162	172	175	177
0070169	145	156	159	162
Mean	149	159	162	163
(±S.E.M.)	3.5	4.0	3.6	4.1

TREATMENT GROUP 6049-3

Animal No.	Day 0	Day 7	Day 10	Day 14
CONTROLS				
Males				
0070127	233	253	257	264
0070134	215	234	235	244
0070138	235	253	260	268
0070143	231	252	253	265
0070147	231	253	255	266
Mean	229	249	252	261
(±S.E.M.)	3.6	3.8	4.4	4.4
Females				
0070154	154	162	162	167
0070156	154	161	163	167
0070160	145	155	152	157
0070175	144	161	155	158
0070176	145	153	155	160
Mean	148	158	157	162
(±S.E.M.)	2.3	1.8	2.2	2.2
TREATED				
Males				
0070116	243	253	257	270
0070117	241	258	263	273
0070122	252	272	276	288
0070131	230	245	247	259
0070141	240	258	261	273
Mean	241	257	261	273
(±S.E.M.)	3.5	4.4	4.7	4.6
Females				
0070150	150	162	161	168
0070153	151	160	157	167
0070161	159	168	165	173
0070163	161	172	170	172
0070177	153	165	160	167
Mean	155	165	163	169
(±S.E.M.)	2.2	2.1	2.2	1.3

TREATMENT GROUP 6049-4

Animal No.	Day 0	Day 7	Day 10	Day 14
CONTROLS				
Males				
0070127	236	253	263	268
0070134	215	234	240	246
0070138	238	256	267	270
0070143	232	255	258	268
0070147	235	253	260	268
Mean	231	250	258	264
(± S.E.M.)	4.2	4.1	4.7	4.5
Females				
0070154	153	162	162	167
0070156	154	165	165	166
0070160	145	156	158	158
0070175	148	158	160	157
0070176	145	152	157	160
Mean	149	159	160	162
(± S.E.M.)	1.9	2.3	1.4	2.1
TREATED				
Males				
0070118	243	260	263	269
0070119	229	248	256	263
0070132	241	260	266	270
0070139	250	270	270	278
0070140	219	234	241	250
Mean	236	254	259	266
(± S.E.M.)	5.5	6.2	5.1	4.7
Females				
0070159	153	162	164	166
0070170	156	167	170	172
0070171	159	172	175	176
0070172	156	168	168	170
0070178	150	164	165	167
Mean	155	167	168	170
(± S.E.M.)	1.5	1.7	2.0	1.8

APPENDIX 9

PRIMARY EYE IRRITATION RESULTS[1] FOLLOWING CONTACT WITH THE FOUR TEST COMPOUNDS

6049-1

Rabbit No.	Washed	Examination Time (Hours Posttreatment) 1	24	48	72
Q05	No	0	20[2]	20[2]	5
Q15	No	2	0	0	5
Q27	No	0	0	20[2]	0
Q31	No	0	0	0	0
Q39	No	2	0	0	0
Q45	No	0	0	0	5
Q53	Yes	0	0	10	0
Q63	Yes	0	0	0	0
Q65	Yes	0	0	0	0

6049-2

Rabbit No.	Washed	Examination Time (Hours Posttreatment) 1	24	48	72
Q03	No	2	5	0	0
Q19	No	0	0	10	5
Q23	No	0	5	0	0
Q35	No	2	0	0	2
Q49	No	2	0	0	10[2]
Q51	No	2	0	20[2]	5
Q61	Yes	0	0	0	10[2]
Q67	Yes	2	12	0	22[2]
Q71	Yes	2	0	5	0

6049-3

Rabbit No.	Washed	Examination Time (Hours Posttreatment) 1	24	48	72
Q09	No	2	0	0	0
Q13	No	2	0	0	0
Q17	No	2	0	10	40[2]
Q25	No	2	40[2]	12	0
Q43	No	2	0	22[2]	7
Q47	No	0	2	2	5
Q57	Yes	0	0	20[2]	0
Q77	Yes	2	0	2	0
Q81	Yes	2	0	0	0

Rabbit No.	Washed	Examination Time (Hours Posttreatment) 1	24	48	72
Q07	No	0	0	10[2]	0
Q11	No	2	4	0	0
Q29	No	2	30[2]	0	0
Q37	No	0	0	0	5
Q41	No	0	5	0	0
Q59	No	0	0	30[2]	0
Q73	Yes	2	0	5	0
Q75	Yes	2	0	0	0
Q79	Yes	2	0	0	0

[1] Combined score for cornea, iris, and conjunctiva effects. Maximum possible value = 110.

[2] High score reflects fluorescein staining of the normal desquamation of the corneal epithelium, considered an aberrant value.

APPENDIX 10

PRIMARY SKIN IRRITATION RESULTS[1] FOLLOWING CONTACT WITH THE TEST COMPOUNDS

6049-1

	Examination Time (Hours Posttreatment)			
Rabbit No.	4	24	48	72
Q05	0	1	0	0
Q15	0	1	1	1
Q27	0	0	0	0
Q39	0	1	1	1
Q45	1	0	1	0
Q53	0	2	0	0

6049-2

	Examination Time (Hours Posttreatment)			
Rabbit No.	4	24	48	72
Q03	0	0	0	0
Q19	0	0	0	0
Q23	0	1	0	0
Q35	0	0	0	0
Q49	0	0	0	0
Q51	0	0	0	0

6049-3

	Examination Time (Hours Posttreatment)			
Rabbit No.	4	24	48	72
Q09	0	0	0	0
Q13	0	0	0	0
Q17	0	0	0	0
Q21	0	0	0	0
Q25	0	0	0	1
Q47	0	1	1	0

6049-4

	Examination Time (Hours Posttreatment)			
Rabbit No.	1	24	48	72
Q11	0	0	0	1
Q41	0	0	0	0
Q73	0	1	0	0
Q75	2	1	1	0
Q79	0	1	0	1
Q81	0	1	0	0

[1] Combined score of erythema, edema, and necrosis. Maximum possible value = 23.

APPENDIX 11

GUINEA PIG 48-HOUR GRADING SCORES

	Test Compound 6049-1		Vehicle	
Animal No.	Erythema	Edema	Erythema	Edema
0070060	0	0	0	0
0070063	0	0	0	0
0070064	0	0	0	0
0070065	0	0	0	0
0070067	0	0	0	0
0070071	0	0	0	0
0070075	0	0	0	0
0070079	1	0	0	0
0070087	0	0	0	0
0070089	2	0	0	0

	Test Compound 6049-2		Vehicle	
Animal No.	Erythema	Edema	Erythema	Edema
0070056	0	0	0	0
0070058	0	0	0	0
0070059	0	0	0	0
0070070	0	0	0	0
0070073	0	0	0	0
0070078	0	0	0	0
0070080	0	0	0	0
0070081	0	0	0	0
0070088	0	0	0	0
0070092	0	0	0	0

	Test Compound 6049-3		Vehicle	
Animal No.	Erythema	Edema	Erythema	Edema
0070055	0	0	0	0
0070061	0	0	0	0
0070069	0	0	0	0
0070072	1	0	0	0
0070074	0	0	0	0
0070083	0	0	0	0
0070086	0	0	0	0
0070091	0	0	0	0
0070093	0	0	0	0
0070102	0	0	0	0

	Test Compound 6049-4		Vehicle	
Animal No.	Erythema	Edema	Erythema	Edema
0070062	0	0	0	0
0070066	0	0	0	0
0070068	0	0	0	0
0070076	0	0	0	0
0070077	0	0	0	0
0070082	0	0	0	0
0070084	0	0	0	0
0070085	0	0	0	0
0070094	0	0	0	0
0070100	0	0	0	0

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